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ORIGINAL PAPER

Better safe than sorry? A Fabaceae species exhibits unfavourable pollen properties for developing bee larvae despite its hidden anthers

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Abstract Empirical evidence suggests that pollen chemistry plays an important role in shaping the pollen host spectra of many bee species. Although the underlying mechanisms are poorly understood, pollen diets of several plant taxa have experimentally been found to impede larval development of unspecialized bees. The pollen of all plant taxa, for which such a detrimental effect on bee larval development has been observed so far, is freely accessible in the flowers and thus easily harvestable for flower visitors, suggesting that this pollen might be chemically protected in order to reduce its loss to pollen-feeding animals. In the present study, we compared larval performance of five solitary bee species on pollen diets of the two Fabaceae species *Onobrychis viciifolia* and *Lotus corniculatus*, which have their anthers concealed inside the flowers, with that on control diets composed of host pollen provisions. As the complex flower morphology of the two Fabaceae species already considerably narrows the spectrum of pollen harvesting bee taxa, which might supersede costly chemical protection of the pollen, we expected bees that usually do not exploit Fabaceae to develop well on *Onobrychis* and *Lotus* pollen diets. Larval survival on the *Onobrychis* pollen diet was successful for all five bee species tested. In contrast, larval survival on the *Lotus* pollen diet was reduced in three species despite the fact that *Lotus* flowers are more difficult to exploit for pollen than *Onobrychis* flowers. We conclude that there is no trade-off between pollen concealment and pollen defence in *Lotus* and that pollen of morphologically complex flowers with a restricted visitor spectrum is not necessarily an easy-to-use nutritional source.

Keywords Apiformes · Bee–flower relationship · Fabaceae · Plant defence · Pollination

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Introduction

Bees exploit a large variety of flowering plants for pollen and nectar, which they store in the brood cells of their nests as food for their larvae (Westrich 1989; Michener 2007). Whereas some bee species are strictly specialized on the pollen of a single plant family or genus ("oligolecty"), others are more generalized and collect the pollen of plants from two or more families ("polylecty") (Cane and Sipes 2006; Müller and Kuhlmann 2008). Recent studies suggest that pollen chemistry contributes to shape the pollen host spectra of bees. Pure pollen diets of *Ranunculus* (Ranunculaceae) and Asteroideae (Asteraceae) negatively affected the larval development of all tested bee species that naturally do not collect these pollens (Levin and Haydak 1957; Guirguis and Brindley 1974; Williams 2003; Praz et al. 2008; Sedivy et al. 2011; Haider et al. 2013), while non-host pollen diets of *Echium* (Boraginaceae) and *Sinapis* (Brassicaceae) prevented the development of some bee species but allowed the development of others (Praz et al. 2008; Sedivy et al. 2011). Reduced survival of bee larvae on non-host pollen diets might be due either to a strong physiological adaptation of the bees to the pollen chemistry

of their specific hosts or to unfavourable chemical properties of the non-host pollen (reviewed in Roulston and Cane 2000 and Praz et al. 2008). In fact, pollen nutritional quality might be poor due to low protein content, lack of essential nutrients or difficult nutrient extractability from within the pollen grains (Herbert et al. 1970; Wille et al. 1985; Suárez-Cervera et al. 1994; Dobson and Peng 1997; Rasmont et al. 2005; Somerville and Nicol 2006; Weiner et al. 2010). Furthermore, the pollen might contain secondary metabolites that intoxicate the bee larvae. A growing number of studies report on the occurrence of secondary metabolites in pollen, sometimes in considerable quantities (Detzel and Wink 1993; Dobson and Bergström 2000; London-Shafir et al. 2003; Kevan and Ebert 2005; Kempf et al. 2010; Reinhard 2011), and concentrations of secondary metabolites may differ substantially between pollen and vegetative tissue (Detzel and Wink 1993; London-Shafir et al. 2003). Natural concentrations of the secondary metabolite ranunculin in pollen did not suffice to severely affect survival of two solitary bee species feeding on non-host pollen of *Ranunculus acris* (Sedivy et al. 2012), whereas natural concentrations of the cyanogenic glycoside amygdalin in pollen of *Prunus dulcis* negatively affected survival of honey bee adults (Kevan and Ebert 2005), and natural concentrations of pyrrolizidine alkaloids in pollen of *Echium vulgare* were lethal to honeybee larvae (Reinhard 2011).

Interestingly, all plant species known so far to exhibit pollen properties that negatively affect bee larval survival (several species of Asteroideae and Cichorioideae, *Echium*, *Ranunculus, Sarcobatus, Sinapis, Stryphnodendron*) have open flowers with freely accessible pollen that can easily be collected by any flower visitor (Levin and Haydak 1957; Loper and Berdel 1980; Williams 2003; Pimentel de Carvalho and Message 2004; Praz et al. 2008; Reinhard 2011; Sedivy et al. 2011). Since the quantitative pollen requirements of bees are enormous (Schlindwein et al. 2005; Müller et al. 2006), plants with freely accessible pollen are at risk that a large proportion of their pollen does not serve pollination but instead ends up in the brood cells of bees, including highly unspecialized ones. In these plants, unfavourable pollen properties might thus serve to reduce excessive pollen loss by narrowing the spectrum of pollen harvesting bees (Praz et al. 2008). In contrast, complex flowers that hide their pollen inside specialized floral structures limit pollen loss by restricting access to those bee taxa that are behaviourally or morphologically adapted to exploit them and serve as more reliable pollinators (Vogel 1993; Harder and Barclay 1994; Müller 1995; Westerkamp 1997a; Westerkamp and Classen-Bockhoff 2005). The synthesis of secondary metabolites as defence chemicals against herbivorous insects has high metabolic costs (Paré and Tumlinson 1999; Bekaert et al. 2012), which applies in particular also for the protection of plant tissue involved in reproduction (Hern and Dorn 2001). Therefore, in complex flowers with hidden anthers, a trade-off between morphological concealment and chemical defence of pollen is likely to occur, which might result in pollen that is less protected chemically than the pollen of flowers with freely accessible anthers.

Examples for morphologically complex flowers are species of the Fabaceae, which conceal their anthers in a "keel" that is formed by the two lowermost petals and which are nearly exclusively visited by bees. In order to collect pollen from Fabaceae flowers, bees have to apply force in combination with sophisticated leg movement patterns (Westerkamp 1997b). As a consequence, unspecialized bees are not able to efficiently harvest pollen on Fabaceae and the spectrum of bee taxa that are able to operate and pollinate Fabaceae flowers is considerably limited (Westerkamp 1993). Due to this reduced visitor spectrum and the putatively high costs of chemical pollen defence, it is reasonable to predict that the pollen of Fabaceae flowers might not, or to a lesser extent, exhibit unfavourable chemical properties.

The present study is intended to be a first step to elucidate possible trade-offs between morphological concealment and chemical defence of pollen. Specifically, we investigated whether the larvae of five species of solitary bees, which naturally do not collect pollen on Fabaceae, are able to successfully develop on pure pollen diets of the two Fabaceae species *Onobrychis viciifolia* and *Lotus corniculatus*, which differ in their floral complexity. As control, we used pollen diets that have been collected by the females of the five bee species. We hypothesized that neither of the two Fabaceae pollen diets impedes larval development, or if it does, that larval survival is higher on the pollen diet of the more complex *Lotus* flowers.

Methods

Study system

Bee species

We chose five bee species belonging to the tribe Osmiini (Apoidea: Megachilidae) to test for their ability to develop on pollen diets of two Fabaceae species, *Lotus corniculatus* Linnaeus and *Onobrychis viciifolia* Scopoli. All five bee species are widespread and common throughout Europe. Three of them are strictly oligolectic (Westrich 1989): *Chelostoma florisomne* (Linnaeus 1758) and *Chelostoma rapunculi* (Lepeletier 1841) exclusively collect pollen on *Ranunculus* spp. (Ranunculaceae) and *Campanula* spp.

(Campanulaceae), respectively, while *Heriades truncorum* (Linnaeus 1758) is specialized on Asteraceae with a clear preference for the subfamily Asteroideae. The two other species, *Osmia bicornis* (Linnaeus 1758) and *Osmia cornuta* (Latreille 1805), are polylectic and harvest pollen from 19 and 8 different plant families, respectively (Haider et al. 2013). Whereas no Fabaceae pollen was recorded in the scopal pollen loads of *O. cornuta*, a very small proportion of Fabaceae pollen was found in the pollen loads of *O. bicornis*, indicating that Fabaceae are occasionally used as pollen hosts by this species. All five bee species nest in a variety of pre-existing cavities such as insect borings in dead wood or hollow plant stalks, which facilitates access and manipulation of brood cell provisions and larvae. The females build several brood cells per nest, which are provisioned with a mixture of pollen and nectar before a single egg is laid on top of each provision. The hatched larva feeds on the pollen–nectar provision and spins a cocoon after having consumed the entire provision. *O. bicornis* and *O. cornuta* complete their development in autumn and overwinter as imagines within their brood cells. The other three species overwinter as pupae (*C. florisomne*) or larvae (*C. rapunculi* and *H. truncorum)* and complete metamorphosis to the adult insect in the following spring or early summer.

Plant species

Lotus corniculatus (hereafter *Lotus*) belongs to the tribe Loteae. It is a perennial herb growing in heaths and grasslands across Europe, Asia and North Africa (Jones and Turkington 1986). *Lotus* flowers exhibit secondary pollen presentation, and they operate in the "piston mechanism" (Westerkamp 1997b): the upper rim of the keel is connate except for a small opening at the keel tip and visiting bees exert pressure on both keel and wings, which leads to a downward movement of the keel. Thereby, a small dose of pollen is released through the opening at the keel tip. To harvest larger amounts of pollen during a single visit, bees have to repeatedly move the wing–keel complex downwards in a pump-like action.

Onobrychis viciifolia (hereafter *Onobrychis*) belongs to the tribe Hedysareae. It is a perennial herb, which is native to southern Central Asia and has been introduced as a forage legume to Europe and North America, where it is now widespread in temperate regions (Hayot Carbonero et al. 2011). *Onobrychis* flowers do not exhibit secondary pollen presentation, and they operate in the "valvular mechanism" (Westerkamp 1997b): the upper rim of the keel is not connate, and when the keel is moved downwards through pressure exerted by the visiting bees, it opens along its total length making the anthers accessible as long as the pressure is maintained.

The flowers of both *Lotus* and *Onobrychis* are exclusively pollinated by bees, which are the only flower visitors able to operate them (Westerkamp 1997). According to the pollen presentation mechanism described above, pollen collection from *Lotus* flowers requires more sophisticated movement patterns by the bees than from *Onobrychis* flowers, which is exemplified by the fact that honeybees and the polylectic *O. bicornis* are able to collect pollen from *Onobrychis* flowers, but appear to be unable to efficiently exploit *Lotus* flowers for pollen (M. Haider and A. Müller, personal observation).

The flowering period of *Lotus* and *Onobrychis* in Central Europe usually extends from May to July or August (Lauber and Wagner 2009). It coincides with the flight period of *C. florisomne* (beginning of May to end of June), *C. rapunculi* (mid-June to end of August), *H. truncorum* (mid-June to mid-September) and *O. bicornis* (beginning of April to mid-July) (Westrich 1989). It usually does not or only marginally overlap with the flight period of *O. cornuta* (March to beginning of May).

Experimental design

Experiments with *C. rapunculi*, *H. truncorum* and *O. bicornis* were conducted in the years 2010 and 2011; those with *C. florisomne* and *O. cornuta* in the year 2012. Pollen of the two Fabaceae species was collected in 2010 and 2011.

Experimental pollen diets

Potted plants of *Lotus* and *Onobrychis* were placed in two strictly separated compartments of a large walk-in cage (12 × 8 × 3.5 m) at the Experimental Research Station of the ETH Zürich at Eschikon. The walk-in cage was covered with gauze and provided with hollow bamboo stalks as bee nesting sites. In each compartment, about 150 individuals of *Megachile rotundata* (Fabricius 1784) (Apoidea: Megachilidae) were released in order to allow them to collect the Fabaceae pollen that was later used in the experiments. *M. rotundata* is a polylectic bee species with a preference for Fabaceae (Westrich 1989). It is native to Europe, but due to its rarity in its native range, cocoons of this species were imported from a commercial bee breeder (JWM Leafcutter Inc) in the USA. Completed nests of *M. rotundata* were taken to the laboratory, carefully opened and the pollen/nectar provisions withdrawn. All *Lotus* and *Onobrychis* provisions collected at one time were carefully mixed in one Petri dish each. As control pollen diets, we used for each of the five tested bee species brood cell provisions collected by the female bees under natural conditions. Completed nests of the five species were collected at previously established nest aggregations

in and around Zürich (Switzerland). Nests were taken to the laboratory and carefully opened. Eggs were removed from the brood cell provisions with a thin pair of tweezers and the provisions withdrawn. All pollen provisions were stored at −20 °C for at least 24 h up to 1 year before use in the experiments. In contrast to the three oligolectic species, for which the composition of the cell provisions was known due to their strict pollen specialization, composition of the control pollen diets for the broadly polylectic species *O. bicornis* and *O. cornuta* was unknown. Depending on body size of the imagines, the following amount of pollen provision was provided for each larva: 90 mg for *C. florisomne*, 70 mg for *C. rapunculi*, 40 mg for *H. truncorum* and 400 mg for *O. bicornis.* Due to a shortage of *Lotus* pollen in 2012, larvae of *O. cornuta* received only 300 mg of pollen diet despite the slightly larger body size of *O. cornuta* compared to *O. bicornis.* Preliminary experiments indicated that this amount is adequate for successful larval development. We are aware that the comparison of larval performance on Fabaceae pollen diets collected by *Megachile rotundata* with that on control pollen diets collected by each species itself might represent a potential bias. Such a bias, however, does not pertain to differences in larval performance between the two Fabaceae pollen diets, which was the main focus of our study.

Egg transfer

Eggs were transferred to artificial brood cells containing a pollen diet of *Lotus, Onobrychis* or the control pollen diet, respectively. Per nest, maximally three eggs were removed. Artificial brood cells were made of blocks of beech wood (4 × 2 × 2 cm for the two larger species *O. bicornis* and *O. cornuta*; 2 × 2 × 1 cm for the smaller species *C. florisomne*, *C. rapunculi* and *H. truncorum*) provided with a drilled burrow (2 cm length, 0.8 cm diameter and 1.3 cm length, 0.4 cm diameter, respectively) open both at the front and on the top. These openings were covered with coverslips attached to the block with transparent adhesive tape to permit free viewing into the burrow. Preliminary trials showed that egg transfer onto the rather fluid pollen provisions collected by *M. rotundata* resulted in a high proportion of drowned eggs. Therefore, prior to egg transfer, the artificial brood cells containing the *Lotus* and *Onobrychis* pollen diets were incubated for 12 h under the same conditions as detailed below in a climate chamber to obtain a firmer consistency comparable to the control pollen diets. This incubation period was not expected to bias our results as it was very short compared with the long incubation period that followed egg transfer (see below). As the cell provisions of many *Megachile* species are rather fluid (Westrich 1989) irrespective of whether they contain Fabaceae pollen or not, and as Fabaceae specialists of other megachilid genera (e.g. *Hoplitis*) have rather firm provisions (A. Müller, unpublished data), fluid provisions as observed in *Megachile rotundata* are unlikely to be an adaptation to specifically digest Fabaceae pollen.

Larval development

After egg transfer, all artificial brood cells were incubated in constant darkness within a climate chamber (E7/2; Conviron, Winnipeg, Canada) under the following conditions: 25 °C for 16 h followed by a gradual reduction in temperature to 10 °C within 4 h followed by a gradual increase back to 25 °C within another 4 h. Relative humidity was constantly held at 60 %. Larval development was checked every second day, and the following developmental stages were recorded: (1) egg hatching, (2) feeding without defecating, (3) feeding and defecating, (4) start of cocoon spinning and (5) completion of cocoon. A cocoon was considered completed upon becoming opaque or as soon as the larva had emptied its gut and stopped moving. Brood cells were kept in the climate chamber until autumn and then stored at 4 °C in constant darkness for overwintering. Individuals of *O. bicornis* were sexed and weighed to the nearest 0.1 mg (AB204; Mettler Toledo, Switzerland) the following spring after metamorphosis to the adult stage had been completed. Individuals of *C. florisomne* and *O. cornuta* were sexed and weighed in autumn before hibernation as black diapausing pupae or as fully developed imagines, respectively. Due to an infestation with *Melittobia acasta* (Hymenoptera: Eulophidae) in 1 year, all diapausing individuals of *C. rapunculi* and *H. truncorum* were killed and their adult mass could not be assessed.

Data analysis

Eggs that did not hatch and larvae that had undoubtedly died from external factors such as mechanical damage, attack by parasitic wasps or chalkbrood were excluded from all analyses. Survival of larvae on the different pollen diets was analysed using Kaplan–Meier survival statistics. The number of days between hatching and completion of the cocoon was considered as "censored data": individuals that died before the completion of the cocoon represented the exact observations for which the event (death) occurred. Those that completed the cocoon and entered diapause, i.e. the survivors, were considered the censored observations and thus withdrawn from survival calculations. Differences between survival distributions were analysed with pairwise log-rank tests implemented in SPSS Statistics 20.0.0 and controlled with false discovery rate (FDR) correction (Benjamini and Hochberg 1995). Differences in development time (time between egg hatching and completion of the cocoon) and adult body mass were analysed with Kruskal–Wallis one-

way analysis of variance, followed by pairwise Mann–Whitney *U* tests and FDR correction. Adult body mass was analysed separately for males and females because females of *O. bicornis* and *O. cornuta* are distinctly larger than males. Due to the low number of females that reached the adult stage, adult body mass was statistically explored for males only. Statistical analyses were conducted with SPSS Statistics 20.0.0 for Macintosh OS X.

Results

A total of 469 transferred eggs hatched. Four larvae died of chalkbrood, six of parasitism by the eulophid *Melittobia acasta*, two were killed by unidentified parasitic wasps, four died of mechanical damage due to handling and four escaped from the brood cells. These larvae were excluded from all analyses.

Larval survival

Larval survival of the *Ranunculus* specialist *C. florisomne* and of the *Campanula* specialist *C. rapunculi* differed significantly between the pollen diets (Kaplan–Meier analysis, log-rank tests: *C. florisomne*: $\chi^2 = 22.018$, $df = 2$, $P < 0.001$; *C. rapunculi*: $\chi^2 = 23.717$, $df = 2$, $P < 0.001$; Table 1, Fig. 1a, b). In both species, survival of larvae reared on the *Lotus* pollen diet was significantly reduced compared with larvae reared on the control pollen diet as well as on the *Onobrychis* pollen diet (pairwise log-rank tests: *C. florisomne*: *Lotus*—control: $P < 0.001$; *Lotus*—*Onobrychis*: $P = 0.001$; *C. rapunculi*: *Lotus*—control: $P = 0.002$; *Lotus*—*Onobrychis*: $P < 0.001$). No significant differences in the survival between larvae reared on the *Onobrychis* pollen diet and the control pollen diet were found (pairwise log-rank tests: *C. florisomne*: $P = 0.280$; *C. rapunculi*: $P = 0.661$).

Larval survival of the Asteraceae specialist *H. truncorum* showed a marginally significant difference between the pollen diets (Kaplan–Meier analysis: $\chi^2 = 5.718$, $df = 2$, $P = 0.057$; Table 1, Fig. 1c); it tended to be reduced on the *Lotus* pollen diet compared with both the control and the *Onobrychis* pollen diets (Table 1).

Larval survival of one pollen generalist, *O. cornuta*, did not differ between the pollen diets (Kaplan–Meier analysis, log-rank test: $\chi^2 = 3.143$, $df = 2$, $P = 0.208$; Table 1, Fig. 1e). Larval survival of the other generalist, *O. bicornis*, differed significantly between the pollen diets (Kaplan–Meier analysis, log-rank test: $\chi^2 = 7.100$, $df = 2$, $P = 0.029$; Table 1, Fig. 1d). Here, survival tended to be reduced on the control pollen diet compared with both the *Lotus* and the *Onobrychis* pollen diets, although post hoc analyses did not detect any significant differences (pairwise log-rank tests: *Lotus*—control: $P = 0.053$; *Onobrychis*—control: $P = 0.066$).

Table 1 Larval survival of five species of solitary bees reared on a pollen diet of *Lotus corniculatus* and *Onobrychis viciifolia* and on a control pollen diet

Bee species	Pollen diet	No. eggs hatched	Surviving larvae		Group heterogeneity	
			No.	%	*P*	groups
C. florisomne	*Lotus*	27	10	37.0	<0.001	a
	Onobrychis	30	24	80.0		b
	Control	29	27	89.7		b
C. rapunculi	*Lotus*	43	6	14.0	<0.001	a
	Onobrychis	40	24	60.0		b
	Control	47	31	66.0		b
H. truncorum	*Lotus*	33	18	54.5	0.057	a
	Onobrychis	34	26	76.5		a
	Control	34	30	88.2		a
O. bicornis	*Lotus*	21	21	100	0.029	a
	Onobrychis	19	19	100		a
	Control	24	20	83.3		a
O. cornuta	*Lotus*	14	14	100	0.208	a
	Onobrychis	30	30	100		a
	Control	23	22	95.7		a

Notes Differences in the survival of each bee species on the three different pollen diets were tested using Kaplan–Meier analysis (post hoc test: pairwise log-rank tests with FDR control). Group heterogeneity: for each bee species, pollen diets sharing the same letter did not differ significantly at $P < 0.05$

Larval development time

Larval development time of *C. florisomne* did not differ significantly between larvae reared on the three pollen diets (Kruskal–Wallis test: $P = 0.560$; Table 1, Fig. 2), whereas development time of the other four bee species was significantly different between the pollen diets (Kruskal–Wallis tests: for all species $P < 0.001$).

In *C. rapunculi*, development time was significantly prolonged on the *Onobrychis* pollen diet compared with the *Lotus* pollen diet (Mann–Whitney *U* test: $U = 114.5$, $P = 0.025$) and the control pollen diet ($U = 702.5$, $P < 0.001$), and it was significantly longer on the *Lotus* pollen diet than on the control pollen diet ($U = 167.5$, $P = 0.001$). In *H. truncorum*, development time was significantly prolonged on the *Onobrychis* pollen diet compared with the control pollen diet (Mann–Whitney *U* test: $U = 677.5$, $P < 0.001$). Development time on the *Lotus* pollen diet did not differ significantly from the two other pollen diets after FDR correction (*Lotus*—*Onobrychis*: $U = 677.5$, $P = 0.047$; *Lotus*—control: $U = 363.5$, $P = 0.039$). In *O. bicornis*, development time was

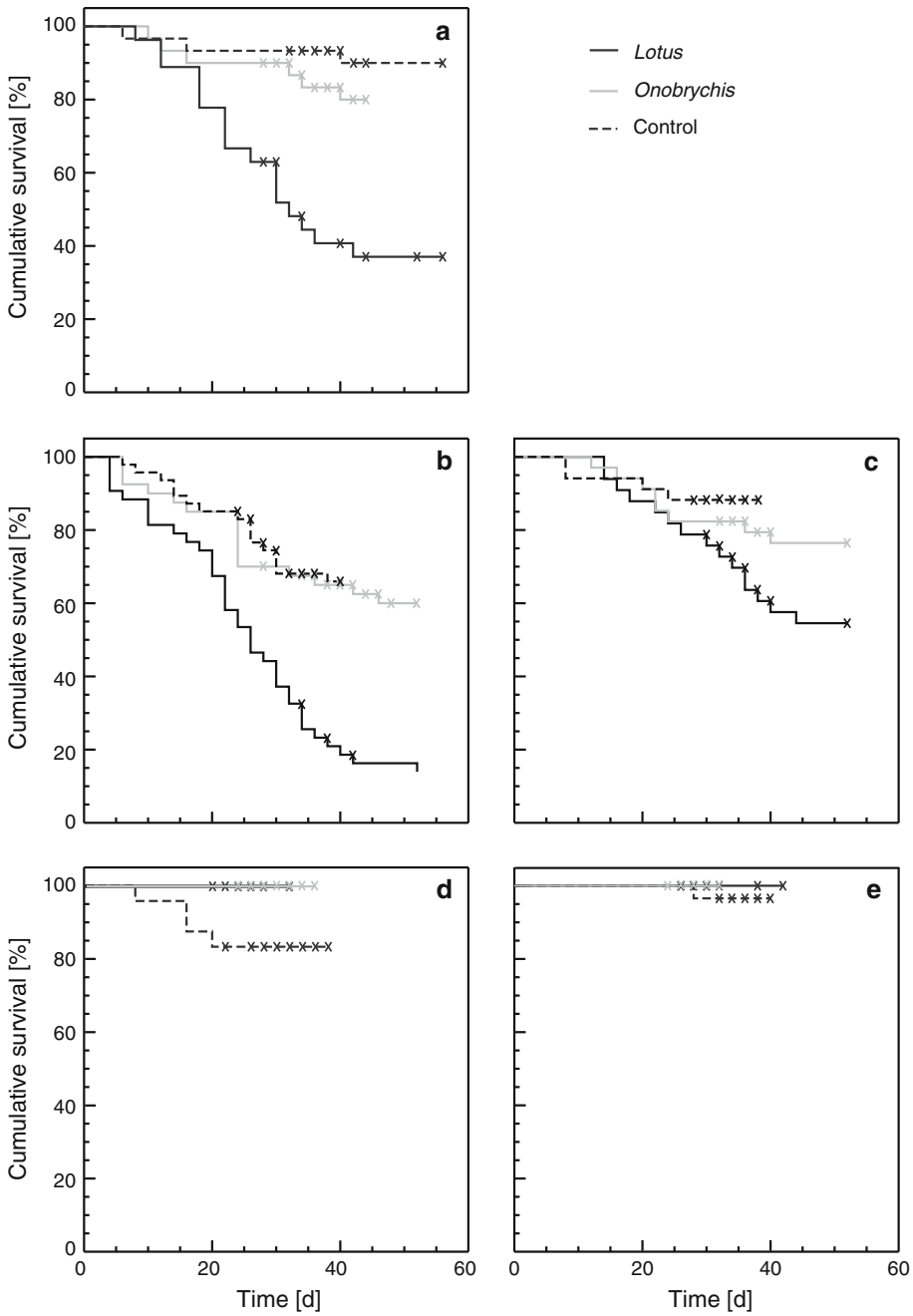

Fig. 1 Cumulative survival of larvae of five solitary bee species when reared on a pollen diet of *Lotus corniculatus* and *Onobrychis viciifolia* and on a control pollen diet that was collected by each species itself. **a** *Chelostoma florisomne*, **b** *Chelostoma rapunculi*, **c** *Heriades truncorum* **d** *Osmia bicornis* and **e** *Osmia cornuta*. *Crosses* indicate individuals that reached the cocoon stage (censored data)

significantly shorter on the *Lotus* pollen diet than on both the *Onobrychis* pollen diet and control pollen diet (Mann–Whitney *U* tests: *Lotus*—*Onobrychis*: $U = 309.5$, $P = 0.002$; *Lotus*—control: $U = 35.0$, $P < 0.001$), and it was significantly shorter on the *Onobrychis* pollen diet than on the control pollen diet ($U = 104.5$, $P = 0.015$). In *O. cornuta*, development time was significantly prolonged on the *Lotus* pollen diet compared with both the *Onobrychis* pollen diet and control pollen diet (Mann–Whitney *U* tests: *Lotus*—*Onobrychis*: $U = 65.0$, $P < 0.001$; *Lotus*—control: $U = 274.0$, $P < 0.001$), and it was significantly longer on the *Onobrychis* pollen diet than on the control pollen diet ($U = 490.0$, $P = 0.002$).

Adult body mass

Male adult body mass of *O. bicornis* did not differ significantly between the pollen diets (Kruskal–Wallis test: $P = 0.155$; Fig. 3b), whereas adult body mass of the other two bee species, *C. florisomne* and *O. cornuta*, was significantly different between the pollen diets (Kruskal–Wallis tests: for both species $P < 0.001$; Fig. 3a, c).

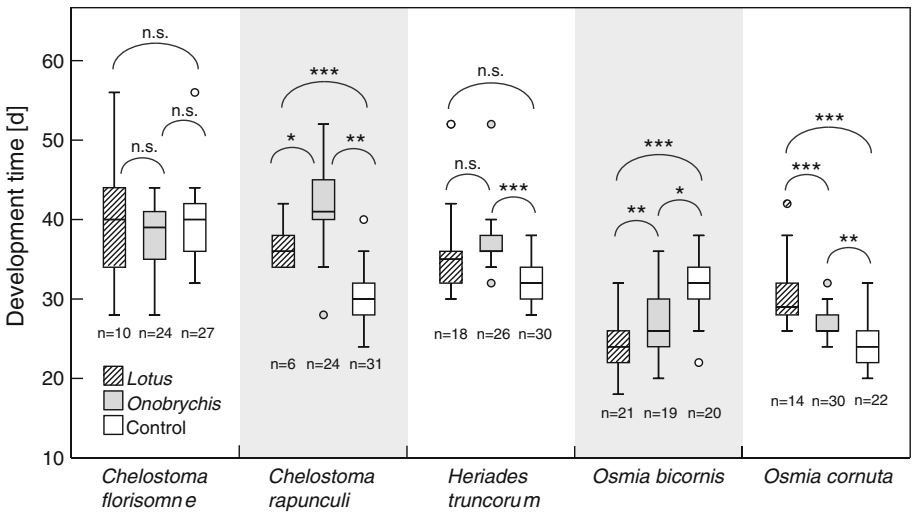

Fig. 2 Development time of larvae of five solitary bee species that successfully reached the cocoon stage when reared on a pollen diet of *Lotus corniculatus* and *Onobrychis viciifolia* and on a control pollen diet that was collected by each species itself. Significant differences are indicated as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) after FDR correction (Kruskal–Wallis test followed by pairwise Mann–Whitney *U* tests)

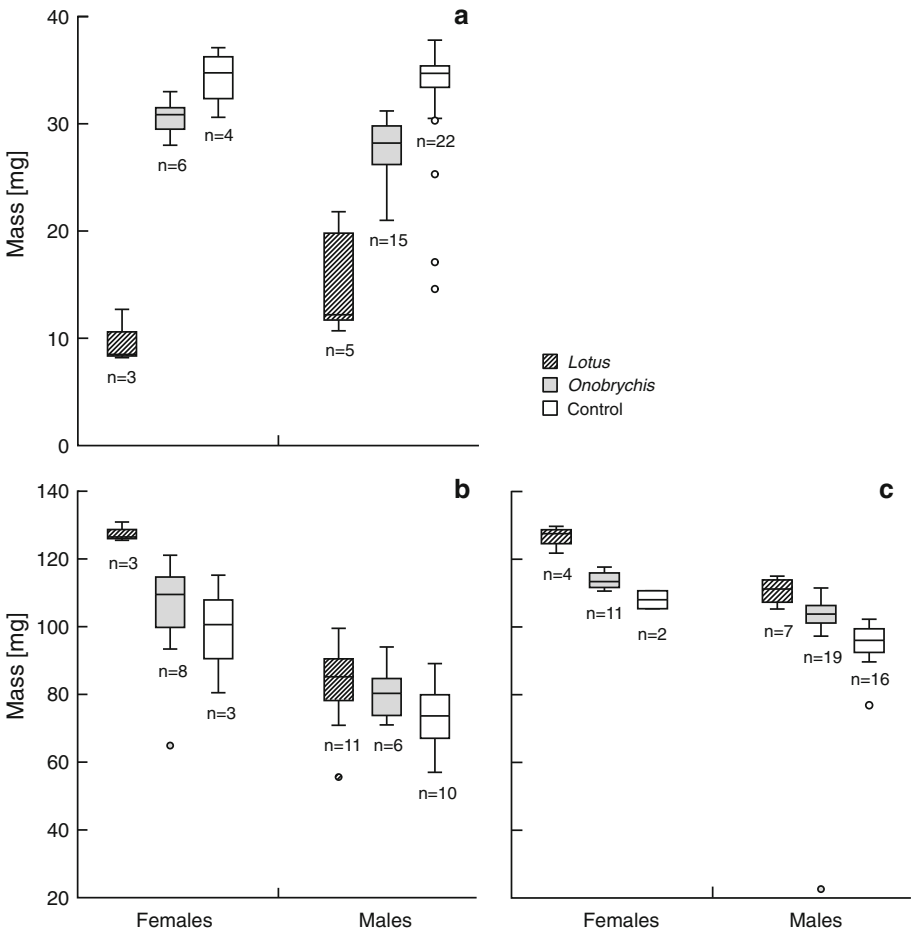

Fig. 3 Adult body mass of **a** *Chelostoma florisomne*, **b** *Osmia bicornis* and **c** *Osmia cornuta* when reared on a pollen diet of *Lotus corniculatus* and *Onobrychis viciifolia* and on a control pollen diet that was collected by the bee species itself

In *C. florisomne*, males reared on the *Lotus* pollen diet had a significantly lower body mass than those reared on the *Onobrychis* pollen diet and the control pollen diet (Mann–Whitney *U* tests: *Lotus*—*Onobrychis*: $U = 74.0$; $P < 0.001$; *Lotus*—control: $U = 106.0$; $P < 0.001$). Males reared on the *Onobrychis* pollen diet had a significantly lower body mass than those reared on the control pollen diet (Mann–Whitney *U* test: $U = 281.5$; $P < 0.001$). In *O. cornuta*, males reared on the *Lotus* pollen diet had a significantly higher body mass than those reared on the

Onobrychis pollen diet and the control pollen diet (Mann–Whitney *U* tests: *Lotus*—*Onobrychis*: $U = 20.0$; $P = 0.006$; *Lotus*—control: $U = 112.0$, $P < 0.001$). Males reared on the *Onobrychis* pollen diet had a significantly higher body mass than those reared on the control pollen diet (Mann–Whitney *U* test: $U = 273.0$, $P < 0.001$).

Discussion

The two Fabaceae pollen diets differed in their suitability as larval food for the tested bee species. All five bee species successfully developed on the *Onobrychis* pollen diet, whereas larval performance on the *Lotus* pollen diet differed among bee species: larval survival was equally successful as on the control pollen diet in the polylectic *Osmia bicornis* and *O. cornuta*, but significantly reduced in the oligolectic *Chelostoma florisomne* and *C. rapunculi*. The oligolectic *Heriades truncorum* tended to perform worse on the *Lotus* pollen diet as well, but this result was only marginally significant. Thus, our initial hypothesis that the pollen of Fabaceae does not exhibit unfavourable properties, which impede larval development of bees that naturally do not collect this pollen, was confirmed for one Fabaceae species. However, it was not supported for the second Fabaceae species, indicating a more complex pattern of interactions between plants with hidden pollen and pollen-feeding flower visitors.

One possible explanation for the reduced survival of the three oligolectic bee species on the *Lotus* pollen diet might be a strong physiological dependency of the specialists upon the pollen chemistry of their specific hosts, which might constrain the ability to utilize alternative pollen. In fact, secondary plant compounds are known to be of nutritional value for some specialized herbivores (Neville and Luckey 1971; Kato 1978; Bernays and Woodhead 1982; Rosenthal et al. 1982; Rosenthal 1983; Slansky 1992 and references therein). However, the successful development of all three oligolectic bee species on the *Onobrychis* pollen diet renders a strong physiological dependency upon the chemistry of their specific pollen hosts rather unlikely. Moreover, *H. truncorum* and *C. florisomne* were experimentally found to thrive well on several types of non-host pollen diets (Praz et al. 2008), suggesting some physiological flexibility to cope with non-host pollen.

Instead, we hypothesize that the increased larval mortality of the three bee species on the *Lotus* pollen diet is attributable to unfavourable chemical properties of the *Lotus* pollen.

As pollen of Fabaceae including *Lotus* has a relatively high protein content and contains all amino acids that are regarded essential for the honeybee (Wille et al. 1985; Roulston et al. 2000; Somerville and Nicol 2006; Hanley et al. 2008), *Lotus* pollen does not seem to be nutritionally deficient. Males of *O. cornuta* even gained more weight when reared on the *Lotus* pollen diet compared with both the control and the *Onobrychis* pollen diet, suggesting a good nutritional quality of *Lotus* pollen. Similarly, as larval development time on the *Lotus* pollen diet was for none of the three bee species prolonged compared with the *Onobrychis* pollen diet, the efficient extraction of nutrients from within the *Lotus* pollen grains does not appear to have been impeded by the pollen wall. If it had been impeded, the surviving bees would have been expected to exhibit a prolonged larval development time in compensation for the reduced nutrient extractability. Thus, we hypothesize that *Lotus* pollen contains secondary metabolites that negatively affected the larvae of the tested bees. This hypothesis is supported by the finding that the larvae of all three oligolectic bee species that managed to develop on the *Lotus* pollen diet stayed relatively small and started to spin their cocoon long before they had consumed the entire provision. This observation might be due to the accumulation of toxic substances in the larval bodies reaching a near lethal threshold. The premature termination of feeding might also explain why the larvae of *C. florisomne* did not exhibit a prolonged development time on the *Lotus* pollen diet despite their significantly reduced adult mass and why *Lotus* reared larvae of *C. rapunculi* and *H. truncorum* exhibited a shorter development time (although marginally not significant in *H. truncorum*) than the *Onobrychis* reared larvae despite their reduced survival.

In contrast to the three oligolectic bee species, larval survival of the two polyleges *O. bicornis* and *O. cornuta* was not negatively affected by the *Lotus* pollen diet. Differences in the survival between generalists and specialists on the same diet might be due to a broader array of detoxification tools owned by the generalists compared with more specialized detoxification tools owned by the specialists, as has been shown for some herbivorous insects (Krieger et al. 1971; Gleadow and Woodrow 2002; Li et al. 2003; Ramsey et al. 2010). Indeed, generalist herbivores might be able to cope with certain plant secondary metabolites that they have never encountered before (Fox et al. 1997; Matsuki et al. 2011; Piskorski et al. 2011), and the ability to digest unfavourable non-host pollen exists in different populations of *O. cornuta* (Haider et al. 2013). However, in a study that compared larval performance of a generalist and a specialist *Osmia* bee species on non-host pollen, larvae of the specialist performed better on the novel pollen than larvae of the generalist, suggesting that specialization does not necessarily reduce the ability of a bee species to use non-host pollen (Williams 2003). Alternatively, the successful development of *O. bicornis* and *O. cornuta* on the *Lotus* pollen diet might result from the possession of specific adaptations to detoxify the

putative secondary pollen metabolites. *O. cornuta* exhibits a strong preference for Rosaceae as pollen hosts and also *O. bicornis* often exploits Rosaceae (Tasei 1973; Haider et al. 2013b), rendering both species highly suitable for the pollination of orchards (Bosch 1994; Krunic and Stanisavljevic 2006; Gruber et al. 2011). Many Rosaceae contain cyanogenic glycosides in their vegetative parts (Conn 1969; Vetter 2000; Wink 2010), and in almond (*Prunus dulcis*) these glycosides have been detected in considerable concentrations also in the pollen (London-Shafir et al. 2003). *O. cornuta* is commercially used for almond pollination, and larval survival on almond pollen provisions is high (Torchio et al. 1987; Bosch 1994), indicating that *O. cornuta* larvae can develop well on a pollen diet that contains cyanogenic glycosides in concentrations that were found to negatively affect the survival of adult honey bees (Kevan and Ebert 2005). Interestingly, many Fabaceae including *Lotus* (but not *Onobrychis*) also possess cyanogenic glycosides in their vegetative parts (Jones and Turkington 1986; Vetter 2000; Wink 2010). Given that such glycosides are also present in the pollen of *Lotus*, the ability of *O. bicornis* and *O. cornuta* to develop on *Lotus* pollen might thus possibly be due to the possession of specific physiological adaptations to detoxify cyanogenic glycosides.

The morphology of *Lotus* flowers, which requires the application of force and a particular movement pattern of the bee in order to efficiently harvest pollen (Westerkamp 1997b), renders pollen collection difficult. In fact, the spectrum of bee taxa that possess behavioural adaptations to collect pollen on *Lotus* and other Fabaceae taxa is considerably restricted, and many bee taxa are unable to efficiently exploit these flowers for pollen and nectar (Westrich 1989; Westerkamp 1993, 1997b). Nevertheless, the present study clearly suggests that the pollen of *Lotus* possesses unfavourable chemical properties for bee larval development. These properties might contribute to further narrow the already limited spectrum of flower visitors or, alternatively, to deter pollen-feeding animals other than bees.

In conclusion, the results of the present study partly contradict the hypothesis that flowers with hidden anthers do not possess unfavourable pollen for bee larval development. In fact, their interactions with pollen-feeding insects appear to be more complex. Whereas pollen of *Onobrychis* supports development of bee species not specialized on Fabaceae, pollen of *Lotus* apparently exhibits unfavourable properties despite the fact that it is more difficult for bees to remove pollen from the *Lotus* flowers than from the *Onobrychis* flowers. Thus, in *Lotus*, no trade-off between pollen concealment and pollen defence could be observed and pollen loss appears to be reduced by a combination of defensive traits that may have evolved under selection from antagonists, be it bees, other pollen consumers or a combination of both.

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